(12) EUROPEAN PATENT APPLICATION

A request for correction of claim 1 has been filed pursuant to Rule 88 EPC. A decision on the request will be taken during the proceedings before the Examining Division (Guidelines for Examination in the EPO, A-V, 2.2).

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(54) Macroporous polymeric membranes, their preparation and their use for polymer separation.

(57) The invention relates to macroporous polymeric membranes, their preparation and their use for polymer separation.

The membranes consist of a cross-linked copolymer on the basis of one or more monovinylic monomers and one or more cross-linking agents and has a globular microstructure whereby the globular structures have a size of 0,05 to 0,5 μm and are mutually interconnected by covalent bonds, and between the globular structures are mutually communicating pores. The thickness is 0,2 to 15 mm, and the specific surface area is up to 400 m²/g.

They are prepared by polymerizing a corresponding monomer mixture including the polymerization initiator in solution in a porogenic solvent in a temperature-controlled mould of appropriate inner dimensions.

The macroporous membranes are particularly suited for the separation of synthetic polymers and biopolymers with gradient elution.

EP 0 320 023 A2

Macroporous polymeric membranes, their preparation and their use for polymer separation

The invention relates to macroporous polymeric membranes, a method for their preparation and to their application to the separation of polymers.

Although the separation of macromolecules from their mixtures with both low-molecular weight and high-molecular weight compounds are very often discussed in the present literature and practice, and considerable improvements could be attained, many problems could not be solved. The problem of an efficient separation of a product from a reaction medium becomes a decisive criterion for a successful method in many modern, fast developing scientific and technical disciplines, e.g., in biotechnology. As a rule. it is not very difficult to find a method for the analytical identification of a desired compound or to determine its concentration. However, difficulties arise by the still incompletely solved problem of preparative industrial separation with high efficiency and a reasonable expenditure of labour, energy, capital and material resources and at minimum environmental load.

The separation and isolation of biopolymers is most important from the aspects of their application. This group of macromolecular compounds comprises particularly oligopeptides and polypeptides, proteins, enzymes, lectins, antibodies, antigens, nucleic acids, and polysaccharides. The separation of biopolymers from natural sources has been the generally known problem already since last century.

The first purification methods were based above all on precipitation, e.g., of proteins, or on salting out with neutral salts, which may be carried out also with simultaneous variation of pH for obtaining even a multiple fractionation in one step. But nevertheless, it was a long way until a pure protein could be obtained, and one of the first successes was in 1926, when Sumner isolated crystalline urease.

The method of chromatography (Ber. Deut. Botan. Ges. $\underline{24}$ (1906) 316) developed in parallel. However, first the work of A.J.P. Martin and R.L.M. Synge (Biochem. J., $\underline{35}$. 1358, 1941), who introduced the concept of the theoretical plate into the liquid chromatography, became an important landmark. It was also stated there that columns packed with microparticles are especially suitable for the separation of macromolecules, the diffusion coefficients of which are very low. Since microparticles were not yet available at that time, the high-performance liquid chromatography (HPLC) was realized first twenty years later.

The chromatography of polymers developed first on the basis of the findings of Peterson and Sober (J. Amer. Chem. Soc. $\underline{76}$ (1954) 1711) that proteins may be adsorbed on the diethylaminoethyl derivative of cellulose and then gradually eluted by a solution with increasing ionic strength. Later-on, also other cellulose derivatives were used for the same purpose, e.g., carboxymethyl cellulose. At the end of the fifties, cross-linked dextran gels were introduced for size-exclusion chromatography (SEC) of proteins and nucleic acids (DE-B-1 292 883; GB-B-974 054), whereby the separation occurs on the basis of the accessability of various portions of the gel structure for macromolecular species of different size. However, the low mechanical strength of gel media did not enable the full realization of the HPLC principle.

A breakthrough occured in the fractionation of bacitracin on microparticles of silica derivatized with alkyl silanes (K. Tsuji, J.H. Robinson. J. Chromatog. $\underline{112}$ (1976) 663). Several structure forms of this peptide were separated by the method of so-called reversed-phase liquid chromatography (RPLC) in a column of a length of 30 cm using an acidic mobile phase containing an organic solvent. Soon after that, papers also appeared describing the application of silica for high-performance size-exclusion chromatography (SEC) and ion-exchange liquid chromatography (IELC) of proteins with almost quantitative yield and retained activity. In comparison with gel materials. Flow rates of the mobile phase almost two orders of magnitude higher can be used in these cases and thus substantially reduce the time of analysis.

Generally, macromolecules interact with the stationary phase in a chromatographic column in various ways. This has the consequence that the capacity factor k' defined for an isocratic elution, i.e. elution using the same solvent all the time, given by

$$k' = (t_R - t_0)/t_0.$$

wherein t_R is the retention time of the desired compound, and t_0 is the column dead time, drastically changes at a slight change in the eluent composition. At certain compositions, k' is so high that the macromolecule practically does not move in the column. However, a small change in the solvent properties causes a decrease in k' to a value close to zero, and the macromolecule passes through the column without any interaction with the packing. The usually measured dependence of log k' on the eluant composition is thus very steep, and often almost vertical. Consequently, the length of the chromatographic column has not a decisive influence on the quality of separation. Very short columns may be used containing only such an

for the detection of the components after separation.

It revealed, that in the chromatography of macromolecules, e.g., by the RPLC method, the resolution function is proportional to

$$D_m^{1/2} \, . \, d_p^{-1} \, . \, t_G^{1/2} \qquad ,$$

where D_m is the diffusion coefficient of the solute, d_p is the diameter of the particles packed into the column, and t_G is the time during which the composition of the solvent mixture varies (gradient). Because the first quantity is a characteristic constant of the separated compound, the quality of separation may be improved by a small change in the solvent composition or by decreasing the size of the particles of the column packing. In the first case, the time necessary for the separation of the mixture increases, whereas in the second case the pressure loss in the column increases, and the eluent has to be introduced under a very high pressure, which may reach several or several tens of MPa.

The given facts show that an enlargement of the scale of a chromatographic separation from an analytical or laboratory scale to an industrial scale brings up immense problems. It is obvious that the application of large-volume columns of a relatively large diameter may cause difficulties, even if incompressible packings are used; this may be seen from the fact that the separation results are not comparable to those obtained on a small scale. The so-called tailing of chromatographic peaks or peak overlapping occur very often. The reason may be the different flow rate of eluent at various sites of the cross-section of column. In order to avoid this effect, a uniform propagation rate of the (horizontal) front has to be attained throughout the column in the direction from the inlet of eluent to the column outlet. This problem is discussed in US A-3 250 058, and for overcoming it breakers fitted inside the column are proposed.

Methods for influencing the flow of liquid in a column are also subject-matter of other patents (US-A-3 539 505; JP-B-73 68 752) which enable to increase the scale of separations, but to the detriment of the original simplicity. In order to overcome the problems involved with complex column design, some authours focussed to the packing as such (US-A-3 856 681) or special methods for column packing (US-A-4 211 656). It revealed later that a suitable effect can be obtained if small particles of the sorbent used for separation are incorporated into a porous inert matrix having a fibrous form. This fibrous material is then packed into a colum of special design, which than exhibits a far lower pressure loss and zone spreading (US-A-4 384 957, 4 512 897, 4 496 461 and 4 604 198).

As indicated above, most of the packings of chromatographic columns for the separation of biopolymers are porous materials on the basis of inorganic polymers (silica gel, glass) or organic polymers (styrene - divinylbenzene, acrylate or methacrylate copolymers, etc.), usually in the form of spherical particles (F.E. Renier, Chromatographia 24 (1987) 241). These particles are prepared predominantly by a suspension technique and, recently, also by the multiste dispersion method (seeded polymerization). On completion of the polymerization, except a few cases, a narrow-size fraction of the sorbent has to be obtained from the raw product, since the quality of the packed column and, consequently, also its efficiency strongly depend on the particle size distribution. The fractionation of particles is very tedious, and the fraction of particles applicable for HPLC is only a small part of the entire quantity of raw product.

In view of the application of small-diameter particles 5 - 10 μm), also the organic sorbents have to be sufficiently mechanically resistant which requires a relatively high concentration of a cross-linking agent in the polymerization batch. The requirement of porosity is met at the same time by the synthesis of macroporous particles, i.e. particles which exhibit porosity also in the dry state or in thermodynamically poor solvents (CS-B-168 268, GB-B-1 512 462), CA-A-1 049 194). From the aspects of morphology, the macroporous polymer particles are characterized by a globular microstructure, i.e. the particles consist of mutually connected submicroscopic spherical entities called globules. The specific surface area of these macroporous polymers is then, as a matter of fact, the surface area of these globules, and the interstitial spaces between them are pores (see e.g., Z. Pelzbauer et al., J. Chromatog. 171 (1979) 101). The globular structure of porous particles suggests to some extent a spherical body filled with small, also spherical globules. Such a suggestion is not far from the conditions occuring inside a packed chromatographic column, which, however, has the shape of a cylinder. Provided the column is packed with particles having the shape of globules of 0,1 to 0,4 μm in size, there will be no practical utility for the chromatographic separation, because the pressure to be applied would be unrealistically high.

Membranes for electrodialysis, which have only the surface layer consisting of a macroporous polymer,

are described e.g. in US-A-3 926 864. Such a surface has, after modification with ionogenic groups, significant anti-fouling properties. Because also suitable electrodialytic properties need to be achieved, the polymerization is carried out in such a way that the inner part of the membranes is microporous (formed by a gel). For this reason, these membranes cannot be used for the separation of polymers. They are suitable for the desalination of water, the removal of ions, and the like.

There are also known plates for thin-layer chromatography, where, however, the sorbent is deposited in the form of a layer on a solid, non-porous substrate (glass, metal). The layer is used in the separation process, which comprises chromatographic separation in tangential direction, i.e., in a path the length of which considerably exceeds the particle size of the sorbent. A further disadvantage of thin layers, which are loosely poured or bonded, is the low resistance to mechanical damage. Accordingly, thin-layer chromatography can be utilized for preparative purposes only with large difficulties.

The above given survey on the state of the art clearly shows that reliable and simple methods for the separation of polymers in larger scale are not available. Therefore, it is the object of the present invention to find a principally new solution and to provide macroporous polymeric membranes suitable above all for the separation of polymers, a method for preparing these membranes and their use, including an apparatus for chromatic separation on the basis of the macroporous polymeric membranes.

The above object is achieved according to the claims. The dependent claims relate to preferred embodiments.

The macroporous polymeric membranes according to the present invention consist of a cross-linked polymer or copolymer on the basis of one or more monovinylic monomers and one or more cross-linking agents which has a globular microstructure whereby the globular structures have a size of 0,05 to 0,5 μm and are mutually interconnected by covalent bonds, and between the globular structures are mutually communicating voids (pores).

According to a preferred embodiment, the macroporous membranes consist of a copolymer of one or more mono vinylic monomers selected from acrylates, methacrylates, itaconates, vinylpyridine, N vinyl pyrrolidone, vinyl acetate, glycidyl vinyl ether, glycidyl vinyl adipate, glycidyl itaconate, glycidyl acrylate, glycidyl methacrylate, and hydroxystyrene and one or more cross-linking agents selected from alkylene diacrylates, particularly ethylene diacrylate, alkylene dimethacrylates, particularly ethylene dimethacrylate, hydroxyalkylene diacrylates, hydroxyalkylene dimethacrylates, divinyl pyridine, and divinyl benzene.

The macroporous membranes according to the invention preferably have a total cross-section thickness of 0.2 to 15 mm, and the specific surface area, which is measurable also in the dry state, is up to 400 m²/g.

The mass ratio of monovinylic monomers(s) and the cross-linking agent(s) is preferably within the range of from 5:95 to 95:5.

The membranes may contain a reinforcing material or insert in order to increase their mechanical strength, preferably throughout the whole cross-section.

Glycidyl methacrylate is advantageously used as the monovinylic monomer in an amount of 5 to 80 % by volume, based on the total volume of monomers including cross-linking agent(s) in the batch, while the residual part of 20 to 95 % by volume is formed by the cross-linking agent and particularly a divinyl monomer, advantageously ethylene dimethacrylate. It is obvious that arbitrary mixtures of both types of monomers may be used, and, in this way, the porosity properties of the formed membranes may be varied.

The monomers and comonomers may be selected within a very broad range. Besides the above listed monovinylic monomers and cross-linking agents, also many other monomers and comonomers can be used. Suitable cross-linking agents may be chosen from diacrylates and dimethacrylates having their vinyl groups connected by ester bonds with a chain of various length or of various hydrophilicity, or from divinylbenzene and others.

The membranes according to the invention may advantageously be produced by
- placing a monomer mixture of one or more monovinylic monomers and one or more cross-linking agents, preferably divinylic monomers, and a polymerization initiator, preferably a radical polymerization initiator, dissolved in a porogenic inert organic solvent in a mould comprising two temperature-controlled, parallel face plates facing each other and a distance insert having a thickness corresponding to the required thickness of the membrane and being adapted to be in a sealing contact with the face plates, whereby size and shape of the inner space of the mould defined by the face plates and the distance insert are adapted to size and shape of the desired membrane,
and
- polymerizing or copolymerizing, respectively, with heating the face plates to the polymerization or copolymerization temperature for the necessary reaction duration.

The polymerization of the mixture in the mould is usually carried out by heating to a temperature of up

monomer, cross-linking agent, and inert porogenic solvent, which increase their size with increasing conversion as far as they come into contact and mutual bonding occurs, thus providing the membrane with the desirable mechanical strength.

Azo-bis(isobutyronitrile) is preferably used in an amount of 0,05 to 2 % by mass, based on the total amount of monomers, in order to initiate the radial polymerization; however, also other radical initiators may be used which may be selected e.g. from azo compounds, peroxides, hydroperoxides, redox systems, and the like.

An important component of the polymerizing system is the porogenic solvent, which is used in the polymerization batch in an amount of 40 to 60 % by volume. Cyclohexanol or its mixtures comprising up to 20 % by volume of dodecanol can be advantageously used. Of course, also other porogenic agents may be used which may be selected from aliphatic and aromatic alcohols, esters, ethers, ketones, hydrocarbons, silicon oils, low-molecular weight polymers, and others.

The polymeric porous membranes prepared according to the invention from reactive monomers can be further modified, and accordingly, their properties can be varied within a broad range. In this way, e.g., the hydrophilicity or lyophilicity may be increased ionogenic groups may be introduced, and catalysts, affinants, or other active groups or molecules can be bound and immobilized.

For example, allyl, amine, sulfonate, hydrogen sulfonate, hydroxy, thiol, and alkyl groups with a chain length of up to 18 carbon atoms may be covalently bound to the inner surface of the membrane by chemical modification.

A fundamental object of this invention is also the application of the above described macroporous membranes, particularly for the separation and fractionation of macromolecular substances and particularly of synthetical polymers and biopolymers.

The general principle of the separation of macromolecular substances on the basis of the macroporous membranes of the invention may be characterized by passing a solution of the macromolecular substances to be separated under pressure through a macroporous polymeric membrane according to one of claims 1 to 8 and 20, with sorption of the macromolecular substances in the membrane, eluting with an elution solvent the properties of which are changed gradually or stepwise in such a manner that individual components of the sorbed macromolecular substances are eluted, and detecting and/or collecting individual eluted components.

In the elution step the pH, the ionic strength, the temperature and/or the composition of the elution solvent are changed, preferably according to a predetermined program.

In accordance with a preferred embodiment, a macroporous polymeric membrane according to one of claims 1 to 8 and 20 is placed and secured in a fixed position on a support member forming a part of the walls of a vessel comprising a liquid chamber provided with means for applying a pressure and eventually also means for stirring above the membrane, and a liquid collection system below the membrane connected to a liquid detection and/or collection system, the solution of the macromolecular substances is then charged into the liquid chamber, pressure is applied to the liquid chamber, and the macromolecular components or fractions are eluted and/or collected.

Usually the pressure is ≤ 1 MPa, while the polymer(s) are sorbed within the membrane. The solvent in which the polymers are dissolved is introduced into the chamber by a pump, and then the properties of the solvent are changed according to the specific program in such a way, that individual components of the sorbed polymer are gradually redissolved and eluted from the membrane and thus are separated. The course of the separation is either only detected with a suitable equiment, and the composition of the polymer or polymer mixture is qualitatively or quantitatively evaluated, or individual fractions are collected yielding the individual components of the separated mixture (preparative separation).

The programmed change of properties of the solvent which causes the gradual dissolution of individual components of the mixture (gradient) may concern the pH, the ionic strength, the content of organic solvent, temperature, and other variables, as mentioned above.

The macroporous polymeric membranes and the method of separation of polymers and particularly of biopolymers according to the present invention have numerous advantages in comparison with the present state of the art. Above all, the membrane preparation is very simple and is not limited with respect to their dimensions. The membranes have sufficient mechanical stability and resistance to physical and chemical effects. The mechanical strength may be further increased, if desired, incorporation of a reinforcing material or insert into the membrane during polymerization. Because the membranes contain reactive groups, they may be readily chemically modified, and functional groups changing their properties may be introduced onto their inner surface, thus substantially enlarging their applicability.

For example, the separation of biopolymers proceeds, in comparison to earlier methods, very fast at a considerably higher load of the separating element than is possible for chromatographic columns. At the

same time, the pressure to be applied in order to obtain the required flow rate is by one to two orders of magnitude lower than in column methods of comparable efficiency. A principal advantage of the membranes and the method of their application according to the invention is, however, the possibility to use theoretically unlimitedly large areas on which the separation of polymers occurs and thus to realize even an

5 industrial process for separating individual macromolecular compounds from mixtures. The required membrane may not only be obtained by one membrane and one chamber, but it is possible to combine several smaller membranes and chambers to blocks having the same desired total membrane area.

The invention is further explained by way of examples.

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Example 1

A mould formed from two metallic plates with drilled communication channels and a distance insert of a square shape and of a thickness of 1,2 mm made from silicon rubber, in which a square window with a side

15 length of 8 cm and a hole enabling to charge the mould space from outside were cut out, was charged with a polymerization mixture consisting of 0,6 g of N-vinyl pyrrolidone, 11,4 g of ethylene diacrylate, 8,2 g of 2-butanone, and 0,15 g of azo bis(isobutyronitrile) (polymerization initiator). Water of 80 °C was led into the mould channels for 8 hours. On completion of the polymerization, the mould was disassembled, and the prepared membrane having a specific surface area of 8,4 m²/g was ready to use. The size of the individual

20 globular structures, as determined by electron microscopy, was 0,48 μm.

Example 2

25 The same mould as in example 1 was charged with a mixture consisting of 0,6 g of glycidyl methacrylate, 11,4 g of ethylene dimethacrylate, 0,12 g of initiator, and 16,2 g of cyclohexanol, and the polymerization was carried out under the same conditions. The prepared membrane had a specific surface area of 139 m²/g; the globular structures had a diameter of 0,05 μm.

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Example 3

A mould, the distance insert of which was 15 mm in thickness, was charged with a mixture consisting of 12 ml of 2-hydroxyethyl acrylate, 12 ml of 2-hydroxypropylene diacrylate, 0,24 g of azo-bis(isobutyronitrile),

35 32,4 ml of cyclohexanol, and 3,6 ml of dodecanol. The polymerization was carried out for 3 hours at 60 °C and then for 5 hours at 80 °C. The obtained polymeric sheet had a specific surface area of 38 m²/g; the monodisperse globules had a diameter of 0,12 μm.

40 ### Example 4

A mould, the distance insert of which had a thickness of 3 mm and was provided with a cut circular window with a radius of 4 cm, was charged with a mixture consisting of 4,8 g of 4-hydroxystyrene, 6,2 g of 2,3-dihydroxybutylene dimethacrylate, 0,15 g of azo-bis(isobutyronitrile), and 12,8 g of methyl benzoate.

45 The polymerization was carried out at 80 °C for 24 hours. The final product in the form of a circular sheet with a diameter of 8 cm had a specific surface area of 12,2 m²/g. The globules had a size of 0,32 μm.

Example 5

50 The same mould as in example 4 was charged with a solution of 7,2 ml of glycidyl methacrylate, 4,8 ml of ethylene dimethacrylate, and, 0,12 g of azo-bis(isobutyronitrile) in a mixture of 16,2 ml of cyclohexanol and 1,8 ml of dodecanol. After 8 hours of polymerization carried out at 70 °C, a membrane was obtained with a specific surface area of 43,3 m²/g, which consisted of globules with a size of 0,16 μm.

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Example 6

A gauze made from polyester fiber with a mesh size of about 300 μm was inserted into a mould comprising a distance insert of 3 mm thickness. The mould, the cavity of which had a square section with a side length of 60 cm, was charged with a mixture containing 19 % of 2-vinylpyridine, 19 % of 2,4-divinylpyridine, 2 % of azo-bis(isobutyronitrile), and 60 % of cyclohexanol. The polymerization was completed at 80 °C after 18 hours, and the resulting product had a specific surface area of 18,9 m²/g.

Example 7

A membrane obtained according to example 2 was immersed into a solution of potassium hydroxide in water with a concentration of 1 mol/l at 60 °C for 18 hours. After washing the membrane with a 0,5 ml/l solution of hydrochloric acid and then with water, it contained carboxylic groups in an amount of 1,4 mmol/g.

Example 8

A membrane obtained according to example 5 was heated for 3 hours in a 0,5 mol/l solution of sulfuric acid. The hydrolytic reaction led to a complete cleavage of the epoxy groups to vicinal hydroxy groups and thus to an increased hydrophilicity of the membrane.

Example 9

A membrane obtained according to example 5 was stirred with a 50 % aqueous solution of trimethylammonium chloride for 10 hours at 80 °C. After washing with a 0,5 mol/l aqueous solution of sodium hydroxide and then with water the content of quaternary ammonium groups determined in the product by titration was 1,93 mmol/l. The product contained 2,70 % of nitrogen according to elemental analysis.

Example 10

A membrane obtained according to example 8 was stirred with a 20 % solution of hexadecanoic acid chloride in benzene. After 6 hours of heating to 60 %, washing with benzene, methanol, and ether, and subsequent drying, the mass of the product was increased by 4,2 %, as compared with the initial mass, which corresponded to a 32 % conversion of epoxy groups originally present.

Example 11

A mould formed from two metallic plates with drilled communication channels and a 1 mm thick distance insert of square shape made from silicon rubber, in which a square window with a side length of 8 cm and a hole enabling to fill the mould from outside were cut out, was charged with a polymerization mixture consisting of 6 ml of glycidyl methacrylate, 6 ml of ethylene dimethacrylate, 0,12 g of azo-bis-(isobutyronitrile) 16,2 ml of cyclohexanol, and 1,8 ml of dodecanol. Water of 70 °C was introduced into the mould channels for 8 hours. After the polymerization was completed the mould was allowed to cool down and disassembled. The obtained sheet of the macroporous membrane was thoroughly washed with alcohol, water and again with alcohol, and allowed to dry. A circular sheet with a diameter of 10 mm was cut out therefrom with a stamp (surface area approx. 300 mm²). The specific surface area of the membrane in the dry state was 62 m²/g.

Examples 12 to 18

The mould described in example 11 was charged with mixtures the composition of which is given in Table 1; the charge was polymerized and worked up in the same way as in example 11.

Table 1

Composition of the polymerization mixture used in the preparation of macroporous polymeric membranes and their specific surface area						
Example No.	GMA (ml)	EDMA (ml)	AIBN (g)	CyOH (ml)	DoOH (ml)	S_g (m^2/g)
12	4,8	7,2	0,12	18	0	80
13	7,2	4,8	0,12	16,2	1,8	45
14	9,6	2,4	0,12	17,1	0,9	160
15	0,6	11,4	0,12	18	0	260
16	7,2	4,8	0,12	18	0	53
17	0,6	11,4	0,06	18	0	250
18	7,2	4,8	0,24	14,4	3,6	35

GMA = glycidyl methacrylate;

EDMA = ethylene dimethacrylate;

AIBN = azo-bis(isobutyronitrile);

CyOH = cyclohexanol;

DoOH = dodecanol; and

S_g = specific surface area in the dry state, determined by the dynamic method of thermal desorption of nitrogen.

Example 19

Circular cut outs from the macroporous membrane prepared according to example 13 were immersed into a 0.01 mol/l solution of NaOH in butanol for 24 hours. After the reaction was completed, the membranes were washed with ethanol and water and stored for further application in the wet state.

Examples 20 and 21

The epoxy groups of the membrane of example 19 were modified with octanol or octadecanol in the same way as in example 19. In the latter case, the modification was carried out at temperature of 65 °C.

Example 22

Circular membrane sheet with a diameter of 10 mm stamped from the membrane of example 16 were immersed for 6 hours into a 25 % commercial aqueous solution of ammonia and heated under reflux to 80 °C. The membranes were then washed with water until no ammonia could be detected in the washing water. The elemental analysis proved the presence of 1,8 mmol/g of amino groups in the modified polymer.

Examples 23 to 30

Sheets from the membrane prepared according to example 11 were modified by the same procedure as in example 22 consisting in the reaction with an amino in pure state or in an aqueous solution carried out in a flask or a sealed ampoule. The reaction conditions and the contents of groups bound to the polymeric membranes are listed in Table 2.

Table 2

Reaction conditions for the modification of macroporous membranes and contents of groups bound to the product					
Example Nr.	Amine	Concentration of aq.solution (% by mass)	Reaction time (h)	Temp. ($^\circ$C)	Content of groups (mmol/g)
23	dimethylamine	100	3	80	2,0
24	2-hydroxyethylamine	100	6	70	2,2
25	bis-2-hydroxyethylamine	100	6	70	2,1
26	octylamine	100	12	70	0,4
27	ethylamine	50	6	80	1,6
28	ethylenediamine	50	10	80	1,7
29	trimethylamine•HCl	50	24	80	2,2
30	tributylamine	100	24	80	0,1

Example 31

Circular sheets cut out from the macroporous membrane prepared according to example 12 were immersed for 6 hours into 0,01 mol/l sulfuric acid and heated to 80 $^\circ$C. The samples were then washed with water until no acid could be determined in the washing water, dipped into a solution of 10,2 g of NaOH in 36 ml of water, and the mixture was cooled down to 0 $^\circ$C. Under stirring of the liquid above the membranes, 24 g of propansultone were added dropwise.

After 30 minutes, the mixture was again heated to 35 $^\circ$C and the reaction continued for further 3 hours. On completion of the reaction, the mixture was allowed to stand at ambient temperature for 12 hours. The samples were washed with water, 0,5 mol/l HCl, and again with water until no acid could be detected in the washing water. In this way sulfo groups were obtained on the surface of polymeric membrane in an amount of 0,85 mmol/g.

Example 32

A rectangular sheet of 10 x 5 cm obtained from the plate prepared in a similar way as in example 12, the epoxy groups of which were hydrolyzed to vicinal dihydroxy groups with diluted sulfuric acid in the same way as in example 31, was washed with water and dried. The dry plate was immersed for 5 hours into a mixture of allyl glycidyl ether and dioxan (1:1 vol/vol) containing 0,3 % by volume of boron fluoride etherate and heated to 50 $^\circ$C. The grafted allyl groups were then modified with a 30 % potassium thiosulfate solution in water at ambient temperature for 24 hours, while oxygen from a cylinder was passed through the mixture every other 10 minutes for 10 minutes. After washing with water, 0,4 mol/l HCl, and again with water, the membrane contained sulfonate groups in an amount of 0,37 mmol/g as determined by acidobasic titration.

Example 33

A circular disk with a diameter of 20 cm cut out from a square plate with a side length of 25 cm and a thickness of 3 mm was immersed into a glass dish containing 100 ml of 1,2-dichloroethane for 20 hours. Then, 45 ml of sulfur trioxide monohydrate was slowly added at room temperature under agitating by tilting the dish. The membrane was removed after one hour of reaction and washed with ethanol and water. Acidobasic titration of a sample revealed that the membrane contained hydrogen sulfate groups in an amount of 1,59 mmol/g.

Example 34

Circular disks with a diameter of 30 mm, which were stamped from the plate prepared according to example 14, were immersed into a 10 % aqueous solution of sodium sulfide. The mixture was moderately shaken at 25 °C for 12 hours. The disks were then washed with water until the odour of hydrogen sulfide disappeared. The modified polymer contained thiol groups in an amount of 0,52 mmol/g.

Example 35

A disk with a diameter of approx. 1 cm cut from a 1-mm thick membrane prepared according to example 11 and modified according to example 19 was placed on the bottom of a vessel with a circular cross-section and a volume of about 1 ml in such a way that, after filling the vessel with liquid, the whole flow occured exclusively through the membrane. The content of the vessel was agitated with a propeller stirrer. Eluent was introduced into the vessel by a pump according to a preselected gradient. Under the membrane, resting on a support surface provided with a system of collection channels which secured a perfect and uniform outlet of eluent from every site of the membrane, also a central outlet capillary opening was provided, from which the passing eluent (or a part thereof) was led into a detector and then could be collected in order to obtain the separated compounds. The vessel was charged with a solution containing 0.1 mg of ribonuclease, 0,1 mg of ovalbumin, and 0,1 mg of chymotrypsinogen in 0,02 mol/l phosphate buffer of pH 6,8 in which ammonium sulfate was dissolved to a concentration of 2 mol/l. The same buffer containing decreasing amounts of ammonium chloride was introduced into the vessel under stirring and a pressure of 0,1 MPa at a flow rate of 0,5 ml/min. The state when the eluent contained 1 % of the original amount of ammonium sulfate was reached after 35 minutes. The eluent flowing from the vessel was led into a Gilson UV detector. The record of the detector response (chromatogram) is shown in figure 111. All originally absorbed proteins were eluted from the membrane in a yield of 100 %.

Example 36

The same mixture of proteins as in example 35 was separated by the same procedure and in the same equipment, but with use of a membrane modified according to example 26. The chromatogram is shown in figure 2.

Example 37

The same proteins as in example 35 were separated by the same procedure and in the same equipment, whereby the amount of each protein in the solution charged into the vessel was 5 mg, i.e. 50 time higher than in example 35. The chromatogram of the separation was absolutely the same as shown in figure 1.

Example 38

A mixture consisting of 0,1 mg of ribonuclease and 0,1 mg of lysozyme dissolved in 0,02 mol/l phosphate buffer of pH 6,8 was separated under the same conditions and in the same equipment as in example 35, but with use of a membrane made according to example 31. The elution was carried out at a flow rate of 1 ml/min and a pressure of 0.1 MPa using a gradient of the ionic strength in the same buffer, whereby the content of sodium chloride was increased so that it reached 0,5 mol/l after 15 minutes. The resulting chromatogram is shown in figure 3.

Example 39

The same mixture of ribonuclease and lysozyme as in example 38 was separated under identical conditions on a membrane prepared according to example 36. The resulting chromatogram is shown in figure 4.

Example 40

A mixture containing 150 mg of ribonuclease, 100 mg of ovalbumin, and 150 mg of chymotrypsinogen was separated in an equipment having the shape of a prism and comprising a chamber with a volume of 10
5 ml with a wall formed by a rectangular membrane with an area of 6 x 8 cm and a thickness of 1,5 mm prepared according to examples 11 and 19. The flow rate of the eluent was 5 ml/min at a pressure of 0.8 MPa. The elution was carried out using the same gradient of ionic strength as in example 38, and the eluent was collected in test tubes in 10 ml portions. The fifth, sixth and seventh test tubes contained 5, 85 and 10 % of the ribonuclease, respectively; the eighth, ninth and tenth test tubes contained 15, 75 and 10 % of the
10 ovalbumin, respectively, and, the twelfth, thirteenth, fourteenth, fifteenth and sixteenth test tubes contained 3, 28, 16, 32 and 20 % of the chymotrypsinogen, respectively.

Example 41

15

A mixture of 0,15 mg of myoglobin and 0,3 mg of ovalbumin was separated on the membrane and in the equipment according to example 35, with the exception that acetonitrile was added into the buffer with decreasing ionic strength in such amount, that its concentration was 12 % by volume after 20 minutes at a pressure of 0,25 MPa. The chromatogram of the separation is shown in figure 5 together with the
20 ammonium sulfate and acetonitrile gradients applied.

Claims

25   1. Microporous polymeric membranes
consisting of a cross-linked polymer or copolymer on the basis of one or more monovinylic monomers and one or more cross-linking agents which has a globular microstructure whereby the globular structures have a size of 0,05 to 0,5 μm and are mutually interconnected by covalent bonds, and between the globular structures are mutually communicating voids.
30   2. Macroporous membranes according to claim 1, characterized in that they consist of a copolymer of one or more monovinylic monomers selected from

acrylates,
methacrylates,
35 itaconates,
vinylpyridine,
N-vinyl pyrrolidone,
vinyl acetate,
glycidyl vinyl ether,
40 glycidyl vinyl adipate,
glycidyl itaconate,
glycidyl acrylate,
glycidyl methacrylate, and
hydroxystyrene,

45

and one or more cross-linking agents selected from alkylene diacrylates, particularly ethylene diacrylate, alkylene dimethacrylates, particularly ethylene dimethacrylate, hydroxyalkylene diacrylates, hydroxyalkylene dimethacrylates, divinyl pyridine and divinyl benzene.
       3. Macroporous membranes according to claim 1 or 2, characterized in that their total cross-section
50 thickness is 0,2 to 15 mm, and the specific surface area measurable also in the dry state is up to 400 m²/g.
       4. Macroporous membranes according to one of claims 1 to 3, characterized in that they consist of a copolymer having a mass ratio of monovinylic monomer(s) and the cross linking agent(s) of from 5:95 to 95:5.
       5. Macroporous membranes according to one of claims 1 to 4, characterized in that they consist of a
55 copolymer comprising glycidyl methacrylate as monovinylic monomer in an amount corresponding to a concentration of 5 to 80 % by volume, based on the total volume of monomers and cross-linking agents present in the polymerization batch.

6. Macroporous membranes according to one of claims 1 to 5, characterized in that they consist of a copolymer comprising ethylene dimethacrylate as cross-linking agent in an amount corresponding to a concentration of 20 to 95 % by volume, based on the total volume of monomers and cross-linking agents present in the polymerization batch.

7. Macroporous membranes according to one of claims 1 to 6, characterized in that the inner surfaces comprise covalently bound allyl, hydroxy, amine, sulfonate, hydrogen sulfonate, thiol and/or C_{1-18}-alkyl groups.

8. Macroporous membranes according to one of claims 1 to 7, characterized in that they contain a reinforcing material or insert.

9. Method for the preparation of macroporous polymeric membranes, particularly those according to one of claims 1 to 8, characterized by
- placing a monomer mixture of one or more monovinylic monomers and one or more cross-linking agents, preferably divinylic monomers, and a polymerization initiator, preferably a radical polymerization initiator, dissolved in a porogenic inert organic solvent in a mould comprising two temperature-controlled, parallel face plates facing each other and a distance insert having a thickness corresponding to the required thickness of the membrane and being adapted to be in a sealing contact with the face plates, whereby size and shape of the inner space of the mould defined by the face plates and the distance insert are adapted to size and shape of the desired membrane,
and
- polymerizing or copolymerizing, respectively, with heating the face plates to the polymerization or copolymerization temperature for the necessary reaction duration.

10. The method according to claim 9, characterized by the use of one or more monovinylic monomers selected from

acrylates,
methacrylates,
itaconates,
vinylpyridine,
N-vinyl pyrrolidone,
vinyl acetate,

glycidyl vinyl ether,
glycidyl vinyl adipate,
glycidyl itaconate,
glycidyl acrylate,
glycidyl methacrylate, and
hydroxystyrene,

and one or more cross-linking agents selected from

alkylene diacrylates,
alkylene dimethacrylates, particularly
ethylene dimethacrylate,
hydroxyalkylene diacrylates,
hydroxyalkylene dimethacrylates,
divinyl pyridine, and
divinyl benzene.

11. The method according to claim 9 or 10, characterized in that the porogenic organic solvent is selected from aliphatic and aromatic alcohols, esters of carboxylic esters, ethers, ketones, hydrocarbons silicon oils, low-molecular weight polymers, and their mixtures.

12. The method according to one of claims 9 to 11, characterized in that the porogenic solvent is used in the copolymerization batch in an amount of 40 to 60 % by volume.

13. The method according to one of claims 9 to 12, characterized in that cyclohexanol or a cyclohexanol/dodecanol mixture comprising up to 20 % by volume of dodecanol is used as porogenic solvent.

14. The method according to one of claims 9 to 13, characterized in that the monovinylic monomer(s) and the cross-linking agent(s) are used in the polymerization batch in a mass ratio of from 5:95 to 95:5.

15. The method according to one of claims 9 to 14, characterized in that glycidyl methacrylate is used as monovinylic monomer in an amount of from 5 to 80 % by volume, based on the total volume of monomers and cross-linking agents present in the polymerization batch.

16. The method according to one of claims 9 to 15, characterized in that ethylene dimethacrylate is used as cross-linking agent in an amount of from 20 to 95 % by volume, based on the total volume of monomers and cross-linking agents present in the polymerization batch.

17. The method according to one of claims 9 to 16, characterized in that the obtained polymeric membranes are chemically modified by introducing ionogenic, hydrophilic and/or lyophilic groups, catalysts and/or affinants and/or other active groups or molecules.

18. The method according to claim 17, characterized in that allyl, hydroxy, amine, sulfonate, hydrogen sulfonate, thiol and/or C_{1-18}-alkyl groups are covalently bound to the inner surface of the membranes.

19. The method according to one of claims 9 to 18, characterized in that a reinforcing material or insert is introduced into the polymerization batch before polymerization.

20. Macroporous polymeric membranes. obtainable according to the method of one of claims 9 to 19.

21. Use of the macroporous polymeric membranes according to one of claims 1 to 8 and 20 for the separation and fractionation of macromolecular substances and particularly of synthetical polymers and biopolymers.

22. Method for the separation of macromolecular substances, characterized by
- passing a solution of the macromolecular substances to be separated under pressure through a macroporous polymeric membrane according to one of claims 1 to 8 and 20, with sorption of the macromolecular substances in the membrane,
- eluting with an elution solvent the properties of which are changed gradually or stepwise in such a manner that individual components of the sorbed macromolecular substances are eluted,
and
- detecting and/or collecting individual eluted components.

23. The method according to claim 22, characterized in that in the elution step the pH, the ionic strength, the temperature and/or the composition of the elution solvent are changed, particularly according to a predetermined program.

24. The method according to claim 22 or 23, characterized in that a macroporous polymeric membrane according to one of claims 1 to 8 and 20 is placed and secured on a support member forming a part of the walls of a vessel comprising a liquid chamber provided with means for applying a pressure and eventually also means for stirring above the membrane, and a liquid collection system below the membrane connected to a liquid detection and/or collection system. the solution of the macromolecular substances is charged into the liquid chamber, pressure is applied to the liquid chamber, and the macromolecular components or fractions are eluted and/or collected.
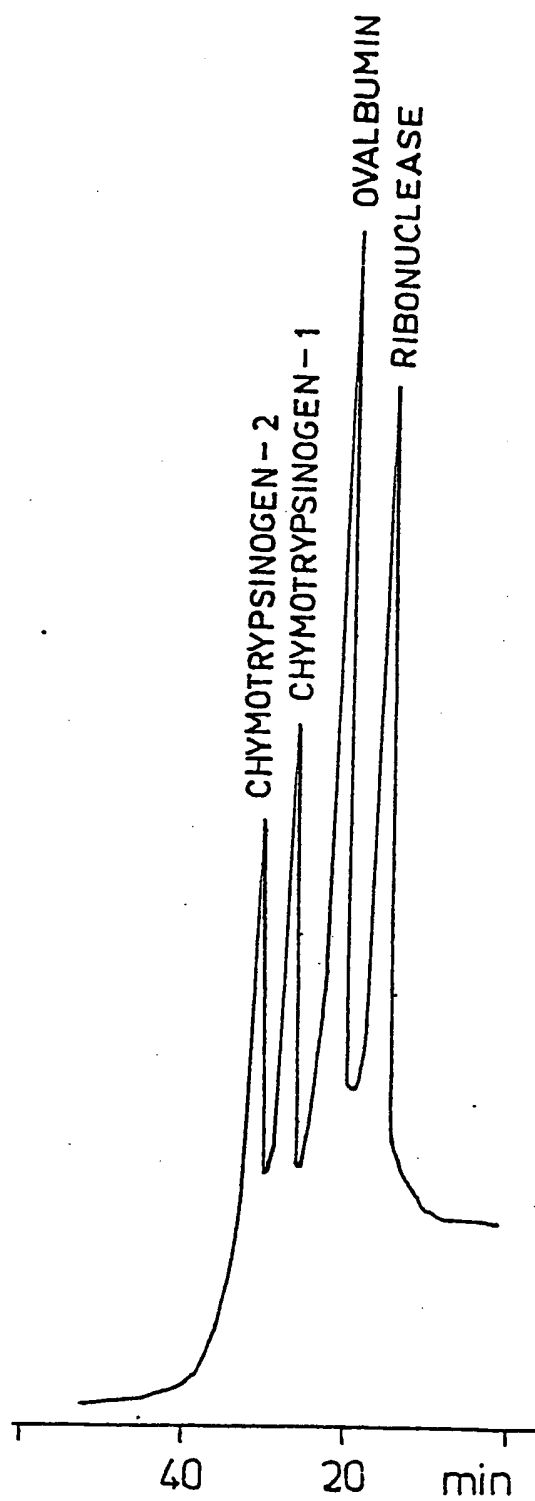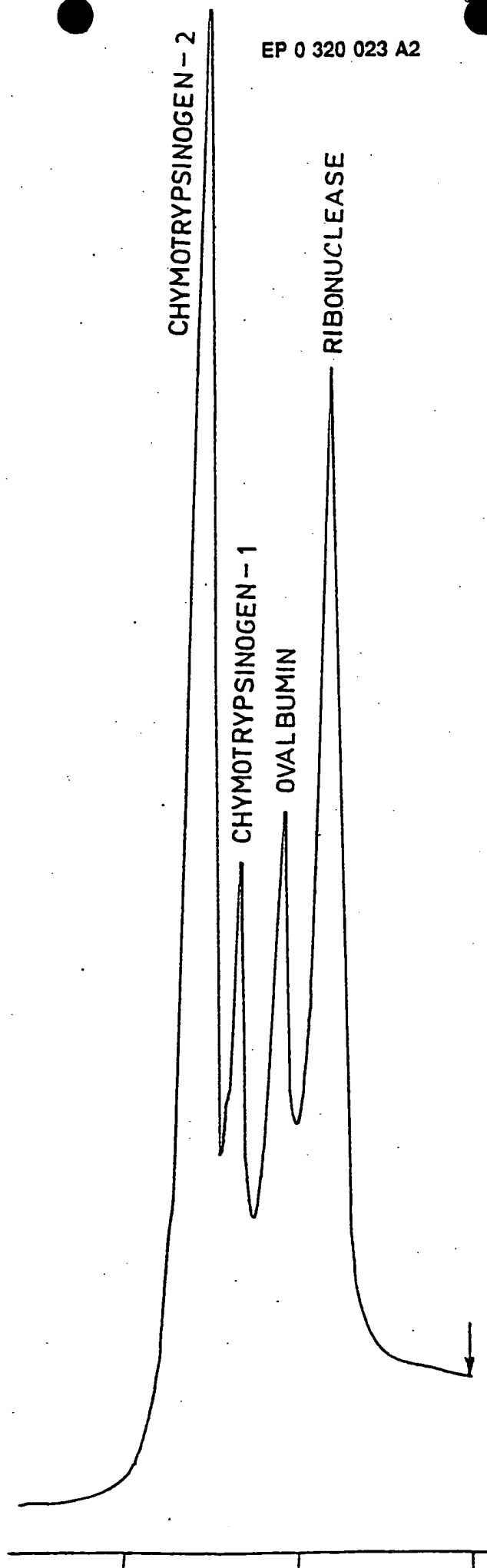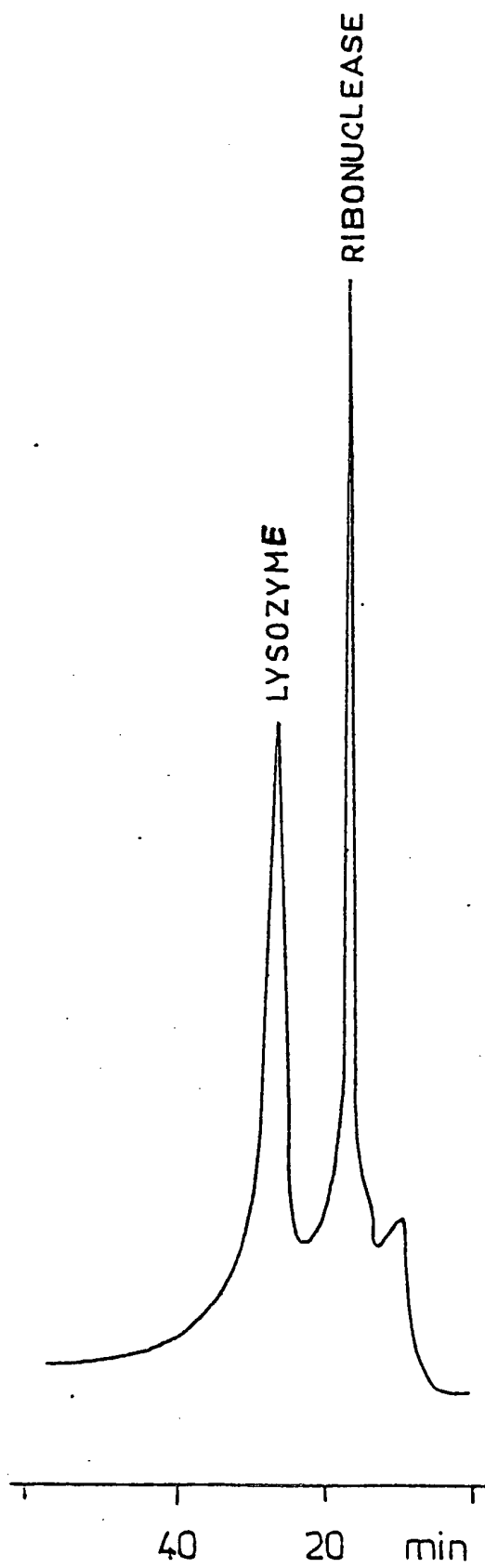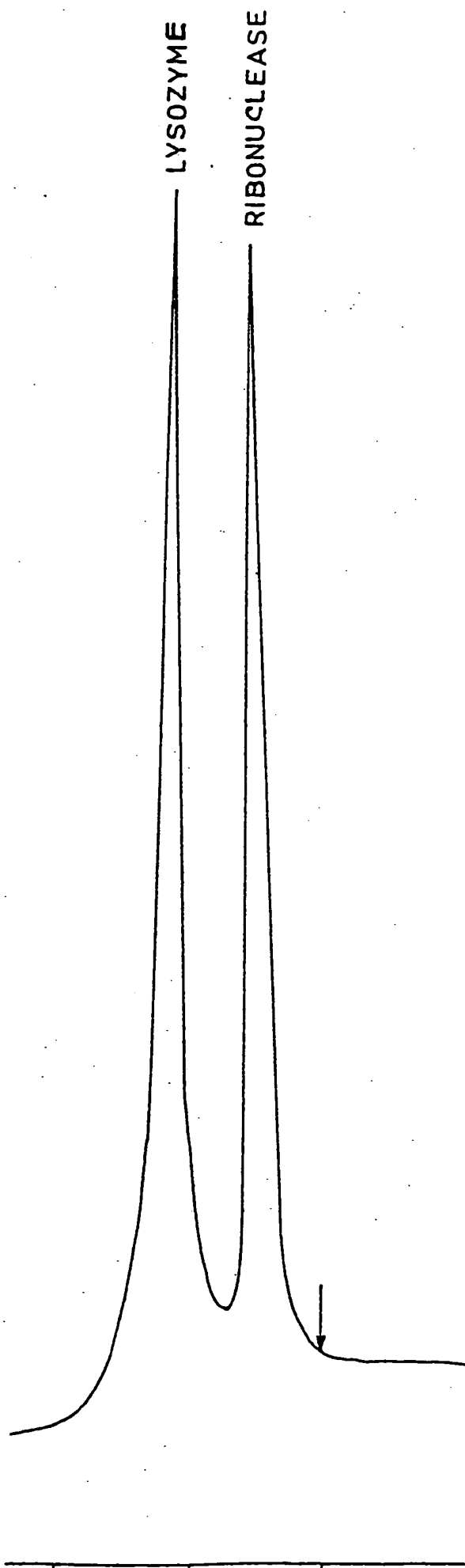
FIG.1

EP 0 320 023 A2

CHYMOTRYPSINOGEN-2

CHYMOTRYPSINOGEN-1

OVALBUMIN

RIBONUCLEASE

FIG.3

protein
(mg)

OVALBUMIN

1.0

MYOGLOBIN

0.5

% CH₃CN

12

0

2   1   0

(NH₄)₂SO₄ Conc. (mol/l)

FIG.5